	SKILLS CENTER **STANDARD OPERATING PROCEDURE**	**A BIOFIZZ** **PRODUCTON**
Protein Expression **Module Hours: 6**	**Effective Date:10/8/2024** **PRQs:** **Skills Center Safety** **Bacterial transformation**	**Revision 1.0** **Author: Zachary Hazlett** **Checked by Editor: M. Stowell**

Background

In biology research, one important approach to understanding a biological system is to purify the individual components of the system and study them *in vitro*. In protein-related research, a key step in the purification and analysis of individual proteins is the step of *protein expression*.

Protein is expressed naturally by living cells as transcription/translation machinery read and produce protein from native DNA. A desirable quantity of protein can be purified from cells naturally synthesizing the protein of interest, a process known as native protein purification, however this process is not well controlled and not very efficient in terms of output. Oftentimes, the quantity of cells needed to reach the desired quantity of protein is too large for this to be used as a regular means of protein purification. This approach is useful in situations where one wishes to purify a protein that is found in complex with a number of other bound proteins. Here the stoichiometry of the individually expressed components is important for the desired outcome. Alternatively, it might be important to purify a protein that is post-transcriptionally modified at the levels naturally produced in the cell. For these reasons and others, one might decide to purify proteins expressed in this manner.

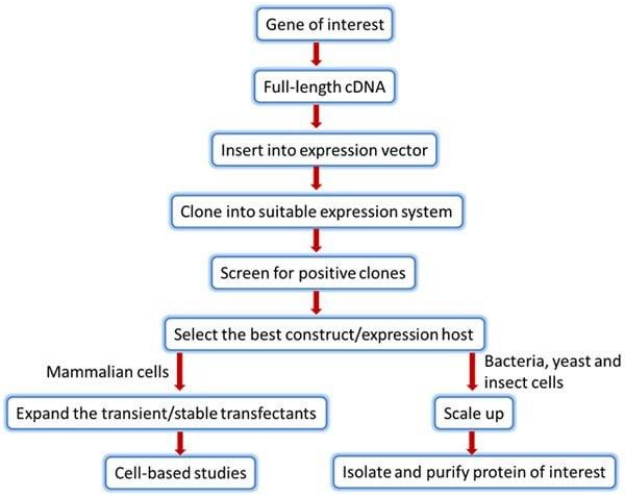


Figure 1. (Source: Sigma Aldrich) The steps of protein purification starting from the gene of interest. In this protocol, we outline the process related to the last two steps (on the right) before protein purification.[1]

If one does not find themselves constrained by research goals such as the above, and simply wants to express and purify large amounts of a single protein, one can express recombinant protein using non-native, recombinant DNA and host cells as the means for protein expression. The gene for the protein of interest is cloned into a plasmid vector and placed under the control of an inducible promotor (see gene cloning SOP). Once the cloned plasmid is purified, it is inserted into a host bacterial cell strain engineered to allow for inducible expression of your gene of interest. After selection for the cells containing your plasmid, these cells can be grown in large quantities. Expression of your protein of interest can be induced at a defined stage in the growth of the bacterial culture and expression of our target gene is accomplished using the bacteria's endogenous protein expression machinery. After the expression step, the bacteria cells, containing large quantities of the target protein (hopefully), can be harvested, tested for proper expression of our protein, and stored until users are ready to purify the protein from the bacteria cells (Figure 2).

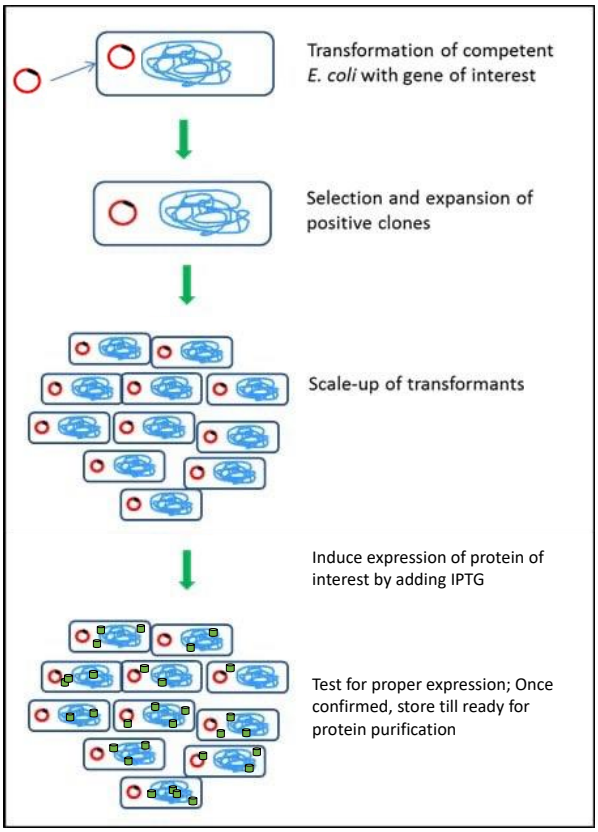
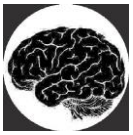


Figure 2. (Adapted from Sigma Aldrich) The steps of recombinant protein expression starting with transformation of purified plasmid (red) into bacterial expression strain. Host genome (blue), expressed protein (green).

	SKILLS CENTER **STANDARD OPERATING PROCEDURE**	**A BIOFIZZ**  **PRODUCTON**
Protein Expression **Module Hours: 6**	**Effective Date:10/8/2024** **PRQs:** **Skills Center Safety** **Bacterial transformation**	**Revision 1.0** **Author: Zachary Hazlett** **Checked by Editor: M. Stowell**

In the following protocol, you will learn how to express the recombinant protein, His6-GFP-TEV (1GFP). This is accomplished using the purified DNA plasmid vector containing the gene of interest and the host bacterial expression strain, BL21.

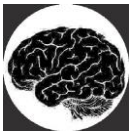
When expressing recombinant protein in this way, one must consider the solubility of their target protein in solution. Many proteins are not stable at room temperature and require expression at lower temperatures to maintain solubility. It is recommended to reference any literature regarding your protein of interest first, if available, to determine the appropriate protein expression parameters. If there is no available information on the solubility of your protein, troubleshooting is likely required to determine which temperature and for how long you must express your protein. In this instance, choose the parameters that are likely to be similar to your protein if possible. In the protocol below, the target protein, 1GFP, is highly thermally stable (up to 70ºC), so expression will be run at 37ºC to maximize bacterial growth and provide proper conditions for 1GFP expression.

Proper protein expression will be monitored throughout the process by collecting small samples of cells at distinct stages of the protein expression process. With each sample run together over SDS-PAGE, one should be able to confirm proper expression and timing of expression of their protein of interest. This is an important quality control step to ensure proper expression prior to the commitment to downstream protein purification steps.

This SOP can be paired with the Bacterial Transformation SOP, as transformation of the plasmid into BL21 cells is the first step. This SOP can also be followed with the protein purification SOP(s), as these preliminary steps are required if no post-expression BL21 cells are available.

Lastly, the following protocol is time intensive. Read through the protocol carefully and plan your experiment according to the time needed for each step.

1. Purpose

	SKILLS CENTER **STANDARD OPERATING PROCEDURE**	**A BIOFIZZ**  **PRODUCTON**
Protein Expression **Module Hours: 6**	**Effective Date:10/8/2024** **PRQs:** **Skills Center Safety** **Bacterial transformation**	**Revision 1.0** **Author: Zachary Hazlett** **Checked by Editor: M. Stowell**

The purpose of this SOP is to instruct students on the background and procedures necessary for expression of the recombinant protein 1GFP using the expression bacterial strain, BL21.

2. Scope

This procedure applies to qualified skills center users.

3. Responsibility

3.1. It is the responsibility of the user to understand and perform the procedure described in this document.
3.2. It is the responsibility of the user performing the procedure to fully document any deviations from the written procedure.
3.3. It is the responsibility of the user to become trained on the procedure.

4. Definitions

4.1 Native protein

Protein translated from the information coded within a cell's own genome.

4.2 Recombinant protein

Protein translated from the information coded within a plasmid containing the recombinant gene of interest. Recombinant protein is expressed using a host cell's protein expression machinery.

4.3 Inducible promotor

A gene promotor that is upstream of our recombinant gene of interest. Seen in the example of the lac operon, expression of the target protein can be controlled by the addition of the inducible sugar, IPTG. Expression is only turned on when IPTG is added to the growing culture of cells.

4.4 Transformation

Introduction of plasmid DNA into a bacterial host cell.

4.5 Bacterial expression strain

A uniform type of bacteria engineered to maximize protein expression. One way this is accomplished is through removal of genes that synthesize proteases.

5. Materials/Equipment

5.0 All materials/equipment for Bacterial Transformation (See associated SOP)
5.1 pET plasmid containing the gene His6-GFP-TEV (1GFP), transformed into BL21(DE3) bacterial expression cells, plated on a Kan plate for selection
(The final product of the Bacterial Transformation SOP).
5.2 LB broth media
5.3 Kanamycin antibiotic, 1000X - 10,000X concentration
5.4 5 ml snap-cap Falcon tubes
5.5 1.5 ml microcentrifuge tubes
5.6 50 mL conical screw-cap polypropylene centrifuge tubes
5.6 1X SDS-running buffer
5.7 Sterile metal wand or toothpicks
5.8 Bactizapper Sterilizer
5.9 37ºC Incubator, rotating or shaking
5.10 2, 250 ml flat-bottomed flasks
5.11 Sterile LB media, 50 mL and 2x100 ml flasks
5.12 IPTG, 100 mM, 1 ml
5.13 Spectrophotometer and clean cuvettes
5.14 SDS-PAGE equipment (See SDS-PAGE SOP)

6. Procedures (Adapted from M. Van Dyke, 2021[2])

6.1 Transform the pET 1GFP plasmid into BL21(DE3) cells
(Follow Bacterial Transformation SOP if needed).
Finish transformation with colonies of cells on Kan plates.
6.2 Inoculate 5 mL LB+Kan in snap-cap Falcon tube with single colony of bacterial cells from plate. Repeat to have two tubes of inoculated LB total.

6.2.1 Add Kanamycin to each 5 ml LB to a final concentration of 1X.
6.2.2 Using a sterilized metal wand or toothpick, pick one colony from plated cells and inoculate one tube of LB+Kan. Sterilize wand and repeat for second tube. Can leave toothpicks or pipette tips in starter culture if they are sterile.

6.3 Incubate tubes in a rack at 37ºC, 240 rpm, for 4hrs to overnight.

6.2.4 Cultures will appear cloudy when growth of starter culture is complete.

6.4 Use each 1 mL culture to inoculate both 100 mL LB+Kan(1X) in 250 ml flat-bottomed flasks.

6.4.1 Culture should be relatively clear upon dilution.

6.5 Incubate 37 °C, 220 rpm, for 2h.

6.6 Cultures should become slightly cloudy. Remove 1 mL aliquot, add to clean cuvette, and measure absorbance at 600 nm (A600). Values are typically 0.2-0.25 at this point. Continue incubation for an additional 30 min. Document time and A600 values.

6.7 Remove 1 mL aliquot and measure absorbance at 600 nm. A600 should be between 0.4-0.5. At this point, cells are in their logarithmic growth (“log”) phase, the optimal stage for inducing expression of target protein.

6.7.1 Take samples for SDS-PAGE. Remove 1 ml from each tube and add to clean 1.5 ml epi tube. After induction and upon next waiting time, centrifuge each for 5 min at 10k. Cells will pellet at bottom of tube. Aspirate or pipette out supernatant leaving the cell pellet undisturbed. Add 100 µl 2X SDS sample buffer (also called 2x Laemmli buffer). Label with corresponding step of procedure and store with all samples to be run over SDS-PAGE.

6.8 Induce expression of target protein.

Important: This step is for only **ONE** of your two samples. We will monitor of expression between these two samples. Label samples clearly as + or – IPTG.

6.8.1 Add stock IPTG (100 mM IPTG, 1000x) to one of the two cultures to achieve 0.1 mM IPTG and continue incubation of both at 37 °C, 220 rpm, for 4h.

6.9 Cultures should have a slight yellow-green hue after 4h induction. Remove 1 mL aliquot and measure A600 (typically 1.8-1.9). Document.

6.9.1 Collect 1ml cells and process sample as described in 6.7.1 above.

6.10 Transfer and split remaining cultures equally between 4 50 mL conical screw-cap polypropylene centrifuge tubes (2/sample, 4 total). Label clearly so that induced and uninduced samples are not mixed

6.11 Centrifuge tubes at 4000 rpm for 10 min at 4 °C.

6.12 Decant supernatant carefully, as pellets may be loose.

6.13 Place labeled aliquots of bacterial pellets in -20ºC for storage.

6.13.1 Label with name, date, type of cells + recombinant plasmid, and a clear indicator the protein that has been expressed w/in these cells.

6.14 Run all test samples containing 1X SDS buffer on SDS-PAGE.

6.15 Document results, clean up.

7. Troubleshooting

7.1 The greatest potential point of challenge in this process is the conditions used for protein expression (i.e. incubation time, expression time, temperature of each step, speed of flask shaking, shape of flask [to allow proper aeration of culture], etc.).

7.1.1 Make sure to take good notes of all steps. If expression is unsuccessful, first consider all steps that have been confirmed to have been done properly, and which might be places of potential mistake.

7.1.2 Bring procedure notes with ideas on what went wrong to proctor and discuss what could be done to improve the procedure next time.

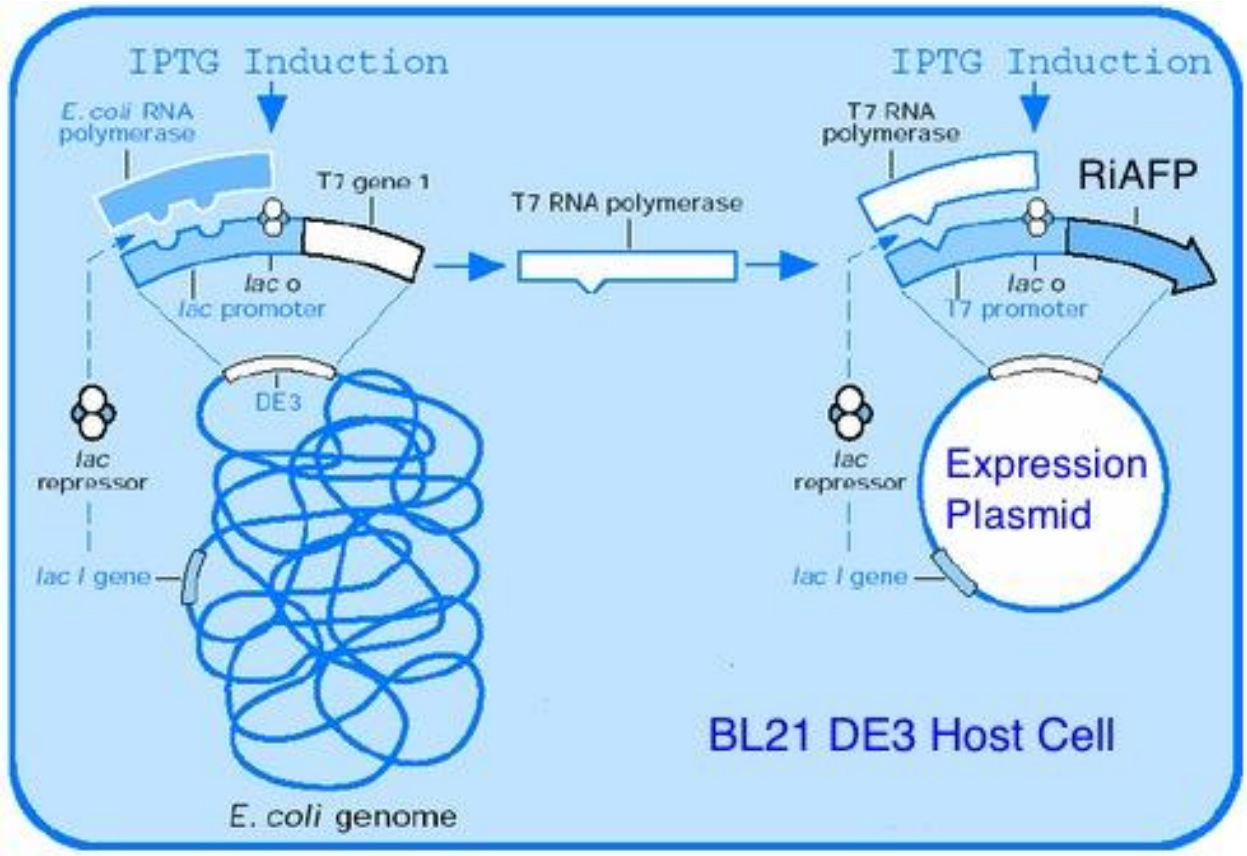
8. References

1. Millipore Sigma. Protein Expression Systems. https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/protein-biology/protein-expression/protein-expression-systems
2. M. Van Dyke. *Expression and Purification of Thermostable Proteins Expressed in E. coli*. 2021. dx.doi.org/10.17504/protocols.io.bq4jmyun

9. Module Methods Task (MMT)

9.1 Briefly explain what recombinant protein expression is and how it is possible.

9.2 Explain how controlled expression of a gene in an expression plasmid under the control of a lac promoter works. Use the below image to help. Be sure to include explanations of the following components: Host cell, expression plasmid, DE3 gene, lac I gene, lac repressor, IPTG, and T7 RNA Polymerase, and T7 promoter.



Source: https://2014hs.igem.org/Team:CSWProteens/project/results

9.3 Grow up 2x100 ml of flasks of bacteria transformed with pET plasmid, inducing expression of one and not the other per the above instructions. Run samples before and after induction together on SDS-PAGE. Using your results, provide proof that expression of your target protein was successful.

10. Feedback

10.1 Please provide a brief description of what you thought was the most helpful components of this SOP

10.2 Briefly describe things you think could be changed to improve this SOP for future students.

10.3 Do the module hour credits appropriately reflect how much time you spent on this skill? If not, did you spend more or less time than the module is worth?

10.4 Submit your results and module mastery task to proctor.